272-Pos Board B72
Role of Serine 58 in the Allosteric Binding Site of E. coli Phosphofructokinase
Bobby W. Laird, Gregory D. Reinhart.
A somewhat unique characteristic of EcPFK is that both of the allosteric ligands, activator MgADP and inhibitor phospho-enol-pyruvate (PEP), bind at the same effector-binding site. This study attempts to understand why two different ligands binding to an identical binding site propagate such markedly different effects. Here we investigated the role that functional groups in PEP play in ligand binding and allosteric propagation via thermodynamic linkage analysis. Previously reported data suggested a potential interaction between PEP and serine 58 in EcPFK contributes substantially to ligand binding and plays a lesser role in allosteric signal propagation. By mutating Ser58 to Ala, Cys, and Asn we have further probed its interaction with PEP. When the Ala, Cys, and Asn mutants were examined, an increase in Kd for PEP of 90x, 30x, and 160x, respectively, that of wild type EcPFK was observed. Each mutant also displays somewhat diminished allosteric inhibition, however allosteric propagation was affected to a lesser degree than binding. In the presence of MgADP the Ala, Cys, and Asn variants exhibit a Kd of 0.25mM, 0.038mM, and 2.8mM, respectively. Comparing the values to wild type EcPFK, with Kd equal to 0.073mM, we see strong changes in Kd while two of the three mutations showed little to no change in allosteric activation. Though these data provide more evidence of a larger role for Ser58 in inhibition relative to activation, no affect on the nature of allosteric phenomena is observed for either MgADP or PEP in the mutants. Also, the examination of inhibitor analogs phosphonoacetic acid, 2-carboxyethylphosphonic acid, and 2-phosphonomethylacrylic acid in the above mutations further strengthens the hypothesis that Ser58 plays a larger role in ligand binding than allosteric coupling. Funding provided by NIH grant GM33216 and the Welch Foundation.

273-Pos Board B73
Adaptative Ligand Induced Folding in an Allosteric Response
Christopher Eginton, Colef Talbert, Dorothy Beckett.
The *Escherichia coli* biotin repressor, BirA, is an allosteric transcriptional regulatory protein that undergoes ligand-induced loop folding upon corepressor binding. The folding results in formation of a hydrophobic cluster composed of a tryptophan and two valine side chains. Functionally, corepressor binding results in enhanced repressor dimerization that is linked to assembly of the transcriptional repressor on DNA. Single alanine replacements in the hydrophobic cluster perturb both corepressor binding and homodimerization, indicating that loop folding is required for the allosteric response. In this work, further perturbation of the hydrophobic cluster was investigated by creating BirA variants with double and triple alanine replacements at these same positions. Isothermal titration calorimetry measurements of corepressor binding and sedimentation equilibrium measurements of homodimerization reveal non-additivity in the effects of the multiply substituted loop. The results further indicate that certain combinations of alanine substitutions lead to reversion of the allosteric response toward that observed for the wild type protein. The reversion may structurally reflect side chain repacking that yields a folded conformation of the loop that is competent for allosteric activation.

274-Pos Board B74
The Intradimeric Alpha1beta1 Interface Holds the Key to Allosteric Control in Hemoglobin
Antonio Tsuneshige, Hajime Nishihara, Takanori Inao, Yuki Yashiro.
The major quaternary structural change responsible for the allosteric transition in human adult hemoglobin (Hb) from low-affinity T- conformation to high-affinity R-conformation upon ligand binding to hemes has been described as a 15-degree rotation of one alpha1beta1 dimer over the pairing one. This interdimeric event has been in the limelight for decades. However, since very few changes, if any, have been reported for the alpha1beta1 intradimeric interface, its contribution to allostery has been neglected. Here, dramatic changes in allosteric characteristics that are caused by a specific steric alteration of the alpha1beta1 interface by chemical modification are reported.
The chemically-modified alpha1beta1 interface Hb showed an extremely low affinity for oxygen and an almost complete absence of cooperativity, comparable to those caused in native Hb by a combination of strong allosteric effectors. The functional characterization is supported by structural evidence revealed by EPR using the nitrosyl derivative, which resembles that one characterized by an extremely low affinity for the ligand showing a triplet superhyperfine around g = 2. Sedimentation experiments showed that modified dimers associated tightly into tetramers. Calorimetric experiments, on the other hand, revealed that the stabilizing interactions were mainly hydrophobic.
The above-mentioned findings suggest that the alpha1beta1 interface is not inert, but rather an extremely important interface playing a pivotal role on the control of oxygen affinity.

275-Pos Board B75
Allosteric Signaling of Anesthetic Isoflurane Bound to LFA-1 Revealed by the PMT Model
David D. Mowrey, Hsiao-Mei Lu, Pei Tang.
Allosteric signaling plays a critical role in biological systems. Mechanistic details of the signaling pathways, however, are often difficult to capture. The Perturbation-based Markovian Transmission (PMT) Model has been developed to mathematically describe the propagation of an initial perturbation through a protein network. Using the PMT model, we investigated how the binding of the anesthetic isoflurane to Lymphocyte Function-Associated Antigen-1 (LFA-1) could affect a remote functional site in LFA-1. The bound isoflurane in the LFA-1 crystal structure (pdb accession: 3F78) was treated as an explicit node in the protein network. Perturbation of the isoflurane node was determined to be consistent with perturbing residues with Cα within 5 Å of isoflurane. We monitored the propagation of the isoflurane-induced perturbation through the protein, examined residues experiencing the top 5% of the perturbation flux, and found that several key residues (S139, L205, and N207) in the metal ion dependent adhesion site and intercellular adhesion molecule-1 binding interface were affected by isoflurane perturbation. Further investigation with the PMT model reveals two paths emanating from the bound isoflurane molecule. Residues identified along both paths experience significant deviation in crystal structure coordinates between open- and closed-conformation LFA-1. The binding of isoflurane seems to have stabilized such a path of contacts that prevent transitions from the closed to open conformation of LFA-1. Our study provided mechanistic details of isoflurane disruption of LFA-1 that are valuable for understanding of the secondary effects of anesthetics on the immune system. Supported by NIH (R01GM66358, R01GM56257, and R37GM049202).

276-Pos Board B76
NMR Investigation of Volatile Anesthetics Interaction with PDZ Domains of PSD-95
Qiang Chen, David Mowrey, Feng Tao, Roger Johns, Pei Tang.
PSD-95 proteins are the most abundant scaffold proteins in the postsynaptic density. PDZ1, PDZ2, and PDZ3 domains (PDZ1-3) are the major components of PSD95. PDZ domains bind to the carboxyl-terminus of PSD-95 partnering receptors, such as NMDA and AMAP receptors. Anesthetics were found to perturb the interaction between PDZ domains and their target proteins. However, it was unclear whether anesthetics interact directly with the PDZ domains. Using NMR, we have investigated anesthetics' effects (such as interaction region and affinity) on the PDZ1-3 domains of PSD-95. 80% of the residues of PDZ123 could be identified with high resolution NMR. Anesthetics (halothane, isoflurane, and sevoflurane) were titrated into the PDZ1-3 samples at 4 ~ 5 different concentrations, which were determined using 19F NMR with trifluoroacetic acid as the external standard. Certain residues showed chemical shift changes in an anesthetic concentration dependent manner in the 15N-1H HSQC spectra. The combined chemical shifts change of 15N and 1H quantified anesthetic perturbation. We found that all three tested anesthetics affected PDZ1-3, but PDZ2 had the most affected residues, while PDZ3 had the least. The affected residues in PDZ1 and PDZ2 were mostly in, or close to, their peptide binding groove, whereas very few residues close to the binding groove in PDZ3 were affected. These findings are valuable for understanding how anesthetics alter signal transduction by perturbing PSD, in particular PDZ1-3 domains. The results are useful for the study of anesthetic perturbation on peptide binding on the PDZ domain with atomic resolution. Supported by NIH (NOT-GM-08-130 and R01GM056257-11).

277-Pos Board B77
Kinetic Enhancement of NF-KB/DNA Dissociation by Ikbalpha
Vera Alverdi, Blake Henderson, Byron Hetrick, Joseph Simpson, Elizabeth A. Komives.
The nuclear factor kappa B (NF-kB) family of transcription factors is involved in inter- and intracellular signaling, cellular stress response, growth, survival, and apoptosis. Specific inhibitors of NF-kB transcription including IkBα, IkBβ, and IkBϵ, block the transcriptional activity of p65 and c-Rel-containing NF-kB dimers. DNA binding by NF-kB is inhibited by the ankyrin repeat protein kappa B (IkBα), which sequesters NF-kB to the cytosol. The mechanism and kinetics of DNA binding inhibition by IkBα are still unknown, but we recently demonstrated that IkBα enhances the dissociation of NF-kB from DNA transcription sites. We are investigating the effect of IkBα on the association and dissociation rates of the NF-kB/DNA complex using titration